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SIGNIFICANCE OF NUTRITIVE CELLS IN INSECT GALLS

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Abstract.—Nutritive cells are modified, highly nutritious plant cells that line the larval chambers of galls and serve as the insect's sole source of food. The development and characteristics of nutritive cells found in the galls of *Urophora cardui* (L.) on *Cirsium arvense* (L.) Scop., *Hemadas nubilipennis* Ashmead on *Vaccinium angustifolium* Aiton and *Diplolepis polita* (Ashmead) on *Rosa acicularis* Lindl. are discussed.

Insects belonging to the gall-inducing guild have evolved the ability to redirect the growth and differentiation of plant cells near the larval feeding sites into structures which provide shelter and a rich food supply. Galls are specific expressions of the insect's biology and there is a vast literature explaining how insects alter the tissues of their hosts (see Mani, 1965; Maresquelle and Meyer, 1965; Rohfritsch and Shorthouse, 1982; Meyer and Maresquelle, 1983).

In contrast to most phytophagous insects which move about the outside of their host organs removing large pieces of plant tissues, gall insects are sedentary and feed on modified plant cells that line the interior surface of their larval chambers. These cells, referred to as 'nutritive cells,' are a characteristic feature of most insect galls (Bronner, 1977). Nutritive cells are usually induced from parenchymatous cells near the feeding sites and not only play a key role in larval nutrition, but also are important in gall development and physiology (Rohfritsch, 1971). The purpose of this paper is to describe the characteristics of nutritive cells and to illustrate the variety of ways in which they are derived from plant cells by examining prosoplasmic galls induced by *Urophora cardui* (L.) (Diptera, Tephritidae) on *Cirsium arvense* (L.) Scop. (Canada thistle), *Hemadas nubilipennis* Ashmead (Hymenoptera, Pteromalidae) on *Vaccinium angustifolium* Aiton (lowbush blueberry) and *Diplolepis polita* (Ashmead) (Hymenoptera, Cynipidae) on *Rosa acicularis* Lindl. (wild rose).

Gall Development and the Characteristics of Nutritive Cells

The series of events whereby insects direct the development of prosoplasmic galls are broken down into three phases: initiation, growth and maturation (Rohfritsch and Shorthouse, 1982). Initiation is the critical period in gall morphogenesis when the insect usurps the host in controlling development of nearby cells. Plant cells are usually in an appropriate state of plasticity for a short period of time (Rohfritsch, 1980) during which initiation must occur. Thus the insect must synchronize its oviposition and feeding within this period.

Gall insects become enveloped by numerous layers of rapidly dividing and enlarging parenchymatous cells during the gall's growth phase. Cells lining the larval chamber develop into nutritive cells at the beginning of the growth phase while adjoining cells remain parenchymatous and accumulate reserves of starch (Bronner, 1977). Cells near the centre of the growing tissues give rise to vascular tissues which join those of the host organ. The maturation phase is marked by a cessation of gall growth; however, differentiation of gall tissues continues. A layer of lignified cells called the hard or sclerenchyma layer appears beyond the starchy reserve layer (Fourcroy and Braun, 1967). Enlarged cortical parenchyma cells beyond the hard layer contain large vacuoles and are thought to act as water storage (Maresquelle and Meyer, 1965). Most larval feeding occurs during the gall's maturation phase.

Nutritive cells in the galls of most insects are characterized by dense cytoplasm, fragmented vacuoles, enlarged nuclei and nucleoli and abundant organelles including ribosomes, plastids and mitochondria. They also contain high concentrations of sugars, proteins and RNA (Bronner, 1977). Cells lining the larval chamber are usually without starch. However, the adjoining parenchymatous cells contain starch in increasing amounts towards the gall periphery. There also is a lipid gradient but it runs opposite to the starch gradient (Bronner, 1977). Thus gall insects, via their nutritive cells, exert a mobilizing effect on their hosts bringing about a relocation of nutrients and in doing so ensure a continuous supply of high quality food. Nutritive cells also play a critical role in the harmonious development of galls and retain their characteristics only if the larvae continue to feed. Death of the larvae due to parasitism, for example, results in a rapid loss of cellular characteristics and further events in gall morphogenesis cease (Rohfritsch, 1971).

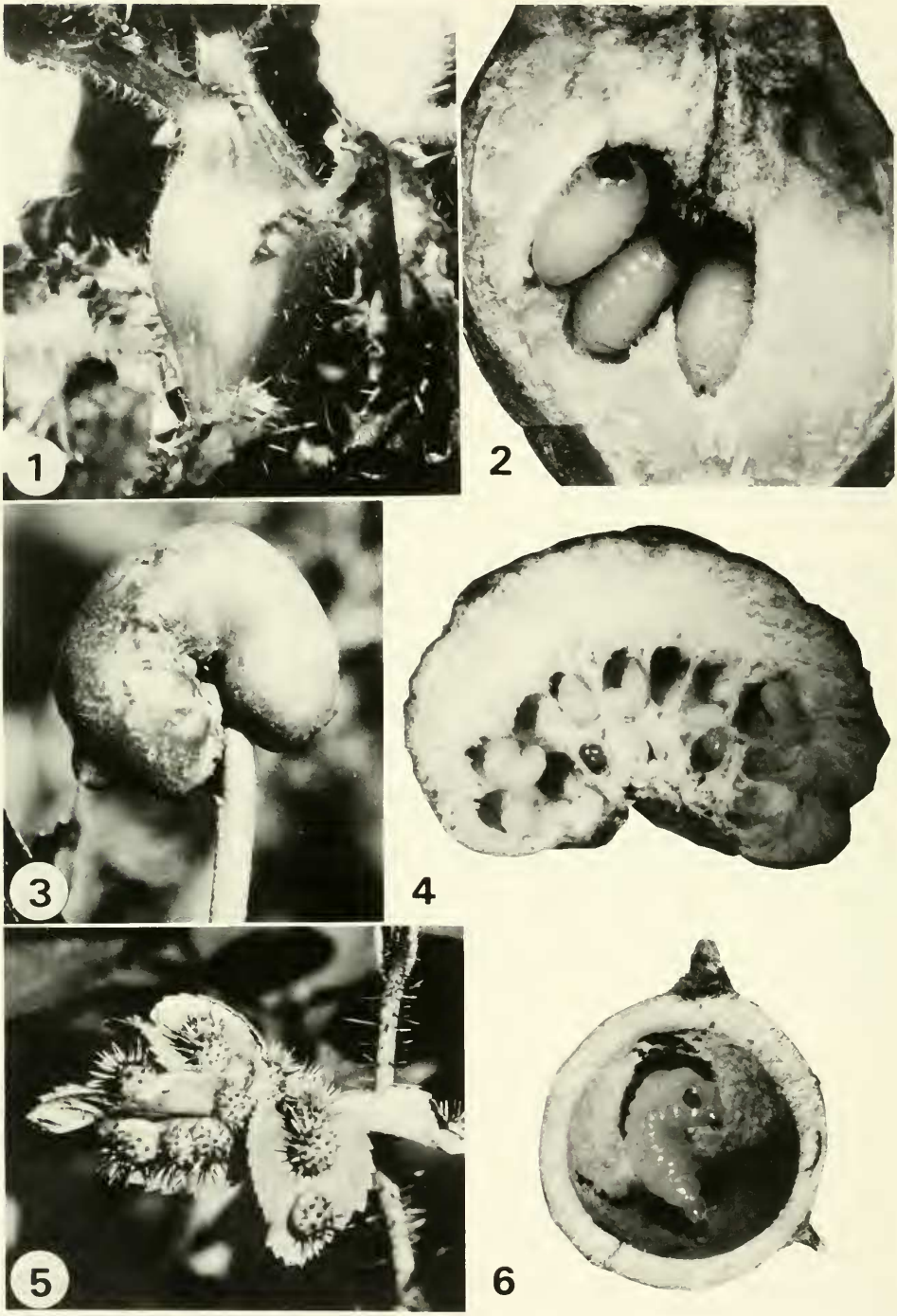
Some authors refer to all tissues found between the larval chamber and the lignified sheath as nutritive tissue since all cells in this region are destined to develop the features of those lining the larval chambers. Bronner (1977) divided the nutritive tissue into two parts, the inner part referred to as 'typical nutritive tissue' and the outer part as 'storage nutritive tissue.' In the present paper, only the cytoplasmically dense cells lining the larval chamber are considered nutritive cells. Cells between the nutritive layer and the lignified sheath are considered gall parenchyma.

Gall of *Urophora cardui* on *Cirsium arvense*

Figs. 1–2, 7–9

Urophora cardui has been extensively studied as a result of its importation from Europe into Canada for biocontrol of Canada thistle (Peschken and Harris, 1975; Lalonde and Shorthouse, 1984). The mature gall is spherical to oblong (Fig. 1) and is induced from stem tissues. It is multi-chambered with each larva in its own chamber (Fig. 2). Structurally the gall is unique since it has two types of nutritive cells referred to as primary and secondary nutritive cells (Lalonde and Shorthouse, 1984).

Eggs are laid in the vegetative shoots and when the second instar larvae hatch, they tunnel into the stems to a region where differentiation of vascular tissue is beginning. Tunnelling produces a wound reaction filling the tunnels with callus thus sealing the larvae within plant tissues. Initiation of the gall begins when pith



Figs. 1–6. 1, Mature gall of *Urophora cardui* on the main stem of Canada thistle (×1.5). 2, Dissection of mature gall of *U. cardui* with fully grown larvae (×6). 3, Mature gall of *Hemadas nubilipennis* on adventitious shoot of lowbush blueberry (×2). 4, Dissection of mature gall of *H. nubilipennis* (×4). 5, Cluster of mature galls of *Diplolepis polita* on the leaflets of wild rose (natural size). 6, Dissection of mature gall of *D. polita* (×8).

and procambial tissues near the larvae begin to proliferate producing gall parenchyma around the larval chambers. As the gall enters the growth phase, gall parenchyma divides rapidly surrounding the larvae with thick masses of cells. Primary nutritive cells are differentiated from gall parenchyma along the base of larval chambers early in the growth phase (Fig. 7). They appear in irregular patches from 2 to 10 cells thick with each cell exhibiting the characteristic dense cytoplasm and enlarged nucleus.

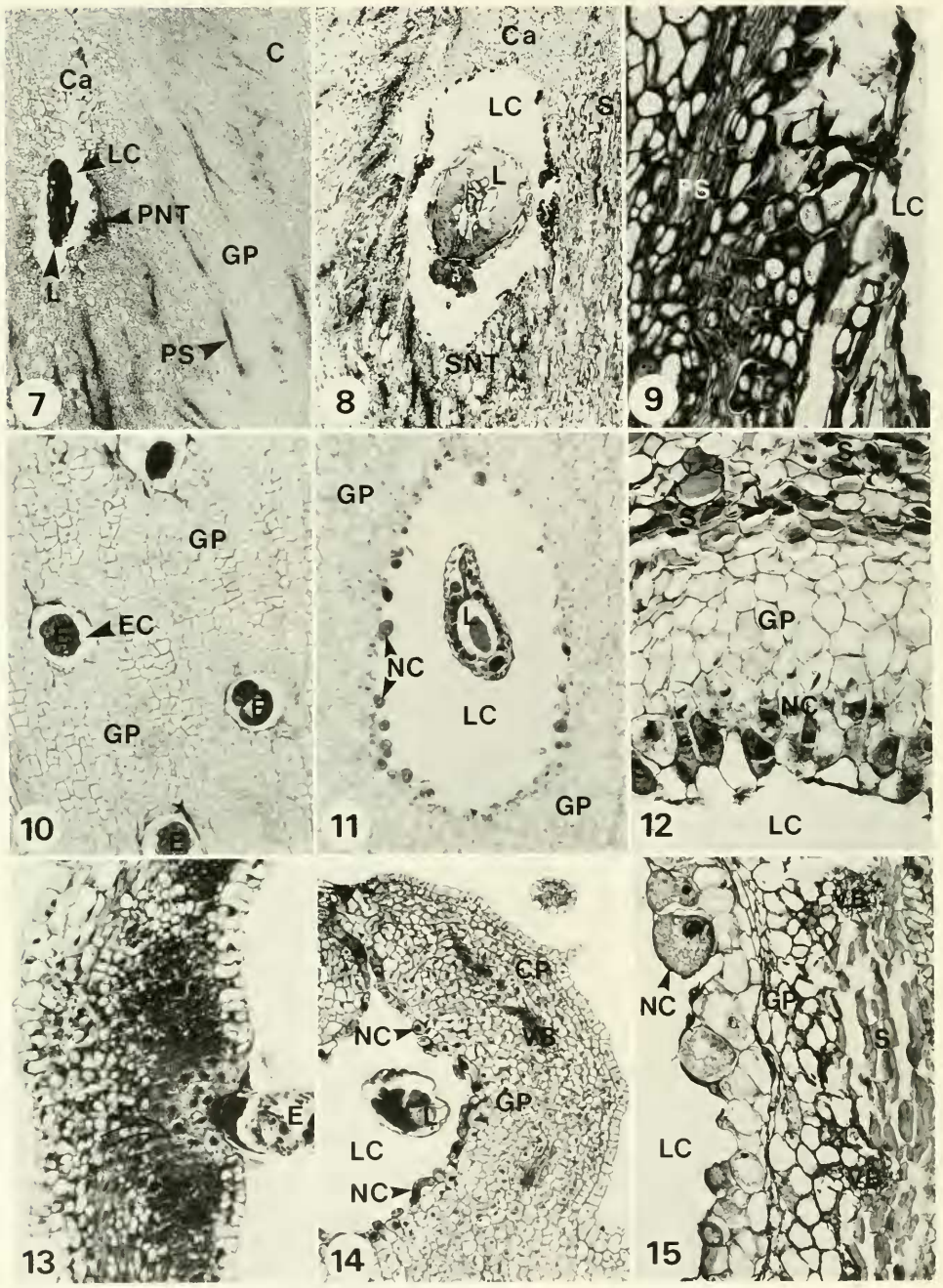
The gall grows rapidly during the growth phase then abruptly slows at the beginning of the maturation phase (Lalonde and Shorthouse, 1985). The sluggish larvae remain in the second instar throughout the growth phase and grow slowly. Once sufficient gall parenchyma has accumulated by the end of the growth phase, the larvae induce the formation of secondary nutritive cells and begin feeding vigorously. The larvae moult to the third instar once secondary nutritive tissue appears. Secondary nutritive cells first appear near procambial strands when primary nutritive cells are still present. However, soon after the onset of gall maturation, all primary nutritive cells are consumed and the larvae are surrounded by secondary nutritive cells (Fig. 8) except for the callus plug at the apex which is to serve as the adult's escape route.

Secondary nutritive cells appear by additive divisions of procambial cells which act as initials. Each derivative cell enlarges tangentially so a gradient of increasing cell size appears away from the procambial initials to the larval chamber (Fig. 9). Secondary nutritive cells have a large central vacuole and an elongate shape; they are rich in protein and lipid-like globules (Lalonde and Shorthouse, 1984). All gall parenchyma beyond the secondary nutritive tissue lignifies. All secondary nutritive cells are consumed by the end of the maturation phase leaving the larval chambers lined with lignified cells (Fig. 2). It appears that the primary nutritive cells are regulatory centres for gall growth and development, whereas the secondary nutritive cells serve to transfer nutrients to the larvae (Lalonde and Shorthouse, 1985). Second instar larvae are essentially non-feeders responsible for directing plant biomass around themselves, whereas the third instar is responsible for food consumption and growth.

Gall of *Hemadas nubilipennis* on *Vaccinium angustifolium*
Figs. 3–4, 10–12

Hemadas nubilipennis Ashmead is the only nearctic member of the family Pteromalidae with the ability to induce galls. Its galls are induced on lowbush blueberry, a deciduous perennial native to northeastern North America. Mature galls are reniform or globular and usually are found at the tips of adventitious shoots (Fig. 3) which arise from buds on rhizomes. Mature galls are multichambered with chambers arranged in rows (Fig. 4); only one larva is found in each chamber.

The life cycle of *H. nubilipennis* has been described by West (1983). Females oviposit into undifferentiated tips of adventitious shoots while the shoots are still within the leaf litter (Shorthouse et al., 1986). Eggs are deposited in vertical rows at the end of oviposition channels and are placed such that they transect the procambial strand distal to the point of entry. Gall initiation occurs before the eggs hatch. The egg stage lasts for approximately 14 days, but during this period tissues surrounding the egg chambers undergo rapid cell divisions. The female



Figs. 7–15. Sections of galls of *Urophora cardui* (7–9), *Hemadas nubilipennis* (10–12) and *Diplolepis polita* (13–15). See Lalonde and Shorthouse (1984) for techniques used in sectioning and staining. 7, Longitudinal section of *U. cardui* gall in early part of growth phase (×25). 8, Longitudinal section of *U. cardui* gall in mid-maturation phase (×23). 9, Longitudinal section of maturing secondary nutritive cells lining larval chamber of *U. cardui* gall (×160). 10, Longitudinal section of adventitious shoot inhabited by eggs of *H. nubilipennis* 10 days after oviposition (×90). 11, Cross section of *H. nubilipennis* gall in early growth phase (×90). 12, Cross section of *H. nubilipennis* gall in maturation phase showing

also stabs the shoot apex after oviposition and growth of the shoot stops. Shorthouse et al. (1986) suggested that larvae and gall tissues become physiological sinks with all nutrients normally destined for shoot growth are instead going to the gall.

Cells surrounding the eggs begin to divide within 48 hours of oviposition becoming the gall parenchyma and, after 10 days, thick layers of uniform, compact gall parenchyma surround each egg (Fig. 10). Cells closest to the eggs are arranged in a radial pattern about the egg chambers (Fig. 10). Freshly hatched larvae begin feeding on gall parenchyma and within 5 days small clumps of nutritive cells are induced along the inside surface of the chambers. Hatching of the eggs approximates the end of the initiation period. Gall parenchyma continues to proliferate and enlarge the gall during the growth phase, which lasts from 60 to 70 days. Nutritive cells, all of which are derived from gall parenchyma, continue to arise and by day 20 form a discontinuous layer around the chamber surface (Fig. 11). The layer is continuous after 40 days. Procambial strands found throughout the gall parenchyma during the growth phase form horizontal anastomoses with the unaffected portion of the vascular cylinder. By the end of the growth phase the nutritive layer is up to 7 cells thick and as the gall enters the maturation phase a sheath of sclerenchyma cells appears lying contiguous to the vascular tissue. The larvae feed most actively during the maturation phase and the zone of parenchyma becomes much reduced (Fig. 12). All nutritive and gall parenchyma are consumed by the end of the maturation phase and the larval chambers become encapsulated by the hard sclerenchyma sheath. Only a thin layer of sclerenchyma separates the larval chambers (Fig. 4) as the mature gall becomes woody and ready for winter.

Gall of *Diplolepis polita* on *Rosa acicularis*
Figs. 5–6, 13–15

Diplolepis polita is one of the most widely distributed of the approximately 28 species of nearctic *Diplolepis*. It has been found across central Canada from British Columbia to Quebec and north to central Yukon and Alaska. Mature galls are spherical and spinulose (Fig. 5) and are found in clusters on the adaxial surface of the leaflets of *Rosa acicularis*. Only one larva is found per gall (Fig. 6). The life cycle and ecology of *D. polita* and other inhabitants of the gall have been described elsewhere (Shorthouse, 1973, 1980).

Eggs of *D. polita* are laid individually in the early spring on leaflets still folded within leaf buds. Only a few epidermal cells are damaged by oviposition along with a small cluster of cells beneath the egg which begin to lyse. Cells next to the lytic zone become highly stimulated within 48 h developing enlarged and fragmented vacuoles. These cells begin to proliferate giving rise to a small pad of tissues (Fig. 13) and as they expand around the lytic tissues they form a slight

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thick layers of nutritive cells lining a larval chamber (×150). 13, Section of egg of *D. polita* and host leaflet 48 hours after oviposition. Note pad of stimulated cells (arrow) beneath the egg (×400). 14, Cross section of *D. polita* gall in growth phase (×115). 15, Cross section of *D. polita* gall in maturation phase (×120). C, cortex; Ca, callus; CP, cortical parenchyma; E, egg; EC, egg chamber; GP, gall parenchyma; L, larva; LC, larval chamber; NC, nutritive cell; PNT, primary nutritive tissue; PS, procambial strands; S, sclerenchyma; SNT, secondary nutritive tissue; VB, vascular bundles.

cavity beneath the egg. The egg hatches within 5 days of oviposition and the larva moves into the cavity.

The initiation phase ends as the larva begins to feed. The larva is surrounded by plant tissues within 8 days of oviposition and soon after cells lining the larval chamber develop into nutritive cells (Fig. 14). A new cambial zone forms within the gall tissues midway between the larval chamber and gall exterior and from these cells gall parenchyma proliferates towards the larval chamber and cortical parenchyma towards the exterior. Gall parenchyma near the larval chamber develops into nutritive tissue, whereas the cortical parenchyma accumulates starch granules. Vascular tissues appear within the cortical tissue (Fig. 14) early in the growth phase and connect with vascular bundles of the host organ. The growth phase lasts for about 20 days.

The maturation phase begins when a layer of cells near the vascular bundles, midway between the larval chamber and gall exterior, begin to lignify. The cortical cells lose their starch granules, but the gall parenchyma continues to give rise to nutritive cells. As the sheath of sclerenchyma cells develops, the gall becomes a series of concentric layers (Fig. 15). Nutritive cells of the maturing gall are the largest cells of the gall (Fig. 15) and have the typically dense cytoplasm and enlarged nuclei. Later in the maturation phase, gall parenchyma inside the sclerenchyma sheath developes into nutritive tissue faster than it can proliferate and thus decreases in thickness. The larva consumes all nutritive cells and gall parenchyma late in the maturation phase, which is about 45 days after oviposition, and the larval chamber becomes lined with the sclerenchyma sheath.

Discussion

The three galls described in this paper are useful models for examining the complex relationships between gall insects and their host plants. They also illustrate how gall ontogeny varies between various groups of gall inducers. Emphasizing the nutritive cells also draws attention to the unique feeding habits of gall insects. No other group of phytophagous insects is able to remain in one feeding site on a host organ and stimulate the plant into supplying it with a steady stream of highly nutritious substances. Nutritive cells are the sole source of food for gall insects and they act as physiological sinks drawing nutrients to the surface of the larval chambers from regions of the plant some distance away. They also influence the composition of nutrients passing through the gall to the larvae.

There are hundreds of insect-induced galls whose structures and the feeding habits of insects within remain unstudied. Perhaps when we determine how more of these systems develop and function, gall studies will make a significant contribution to our understanding of the organic relationships between plant and animal cells.

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